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# Habitual dietary fibre intake influences gut microbiota response to an inulin-type fructan prebiotic: a randomised, double-blind, placebo-controlled, cross-over, human intervention study

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## ABSTRACT

Dysbiotic gut microbiota have been implicated in human disease. Diet-based therapeutic strategies have been utilised to manipulate the gut microbiota towards a more favourable profile. However, it has been demonstrated that large inter-individual variability exists in gut microbiota response to a dietary intervention. The primary objective of this study was to investigate whether habitually low (LDF) *versus* high dietary fibre (HDF) intakes influence gut microbiota response to an inulin-type fructan prebiotic. In this randomised, double-blind, placebo-controlled, cross-over study, 34 healthy participants were classified as LDF or HDF consumers. Gut microbiota composition (16S rRNA bacterial gene sequencing) and short-chain fatty acid concentrations were assessed following 3 weeks of daily prebiotic supplementation (Beneo Orafit<sup>®</sup> Synergy 1; 16 g/d) or placebo (Roquette Glucidex<sup>®</sup> 29 Premium; 16 g/d) as well as after 3 weeks of the alternative intervention, following a 3-week washout period. In the LDF group, the prebiotic intervention led to an increase in *Bifidobacterium* ( $p = 0.001$ ). In the HDF group, the prebiotic intervention led to an increase in *Bifidobacterium* ( $p < 0.001$ ) and *Faecalibacterium* ( $p = 0.010$ ) and decreases in *Coprococcus* ( $p = 0.010$ ), *Dorea* ( $p = 0.043$ ) and *Ruminococcus* (*Lachnospiraceae* family) ( $p = 0.032$ ). This study demonstrates that those with HDF intakes have a greater gut microbiota response and are therefore more likely to benefit from an inulin-type fructan prebiotic than those with LDF intakes. Future studies aiming to modulate the gut microbiota and improve host health, using an inulin-type fructan prebiotic, should take habitual dietary fibre intake into account.

## INTRODUCTION

The commensal microbes that reside within the gastrointestinal tract are implicated in human health and disease. Host genetics (1), life stage, geographical location (2), gender (3) and antibiotic use (4) influence gut microbiota composition; however, diet plays a major role in modulating the community of microbes that reside within the gut (5). Dietary interventions provide an opportunity to manipulate the commensal bacteria towards a more favourable profile to help enhance human health.

Numerous studies have demonstrated that dietary interventions can elicit significant changes in gut microbiota composition and short-chain fatty acid (SCFA) production. In a recent study, a short-term plant-based diet high in grains, legumes, fruits and vegetables or an animal-based diet composed of meat, eggs and cheese led to distinct shifts in bacterial

relative abundance. Interestingly, the animal-based diet had a larger effect on the gut microbiota than the plant-based diet (6). Another study demonstrated that a three-week intervention containing high levels of wholegrains or red meat altered numerous bacterial taxa, including *Collinsella aerofaciens* and certain *Clostridium* spp., and led to an increase in microbial diversity during the high wholegrain dietary phase (7). Dietary intake in economically-developed countries is characterised by intakes of dietary fibre well below recommendations, thus depriving the gut microbiota of valuable fermentable substrates (8,9). One method of enriching the diet to positively modulate the gut microbiota is to supplement it with prebiotics. Prebiotics are “a substrate that is selectively utilized by host microorganisms conferring a health benefit” (10).

It is becoming increasingly evident that there is profound inter-individual variability in gut microbiota response to dietary interventions. Preliminary research has suggested that factors such as microbial diversity, baseline bifidobacteria concentrations and habitual diet are implicated in gut microbiota responsiveness. A study undertaken by Tap and co-authors (11) demonstrated that a short-term alteration in dietary fibre intake in 19 healthy adults led to differing microbial responses among participants. Participants with higher baseline microbial richness had gut microbiota that were more resilient to change and, therefore, less responsive to the change in dietary fibre intake. Several studies have also established a link between baseline bifidobacteria concentrations and change in bifidobacteria in response to a dietary intervention (12–16). Increases in bifidobacteria concentrations are more pronounced in individuals with lower baseline bifidobacteria compared to individuals with higher baseline bifidobacteria concentrations. Preliminary research has shown that habitual diet may also influence gut microbiota responsiveness (17,18). AA 21-day palm date intervention did not influence the numbers of select bacterial taxa, however, secondary analysis demonstrated that those with HDF intakes hosted microbiota that were more stable in response to the palm date intervention than those with LDF intakes (17).

To date, no human studies have been conducted with the primary aim of determining whether habitual dietary intake influences gut microbiota responsiveness to a dietary intervention. Therefore, we aimed to investigate the influence of differing habitual dietary fibre intakes on the responsiveness of the gut microbiota to an inulin-type fructan prebiotic.

## METHODS

This randomised, double-blind, placebo-controlled, cross-over, human intervention study was conducted at Massey University, Palmerston North, New Zealand between March and August 2016. The Massey University Human Ethics Committee approved the study (Massey University HEC: Southern A application- 15/34). The study is registered in the Australian New Zealand Clinical trials registry (ACTRN12615000922572). The study protocol has previously been published (19).

### Participants

Participants were recruited through email and poster advertisement around Palmerston North, New Zealand. A total of forty-four eligible participants provided written informed consent to participate in this human intervention study (**Figure 1**). Participants completed a screening questionnaire to ensure they met the following inclusion criteria: aged between 19 and 65 years; BMI between 18.5 and 30 kg/m<sup>2</sup>; healthy (self-reported and confirmed by a health screening blood test [liver and kidney function, blood glucose levels, electrolytes, complete blood count, calcium and C reactive protein] using standard clinical cut-offs). Exclusion criteria included: a significant change in weight ( $\pm$  5% of total body weight) or dietary intake over the past year; taken antibiotics within the past 6 months; consumption of supplementary prebiotics or probiotic containing foods, drinks and supplements within the past 1 month; pregnancy or breastfeeding (or plans for a pregnancy within the following 3 months); food intolerances associated with gastrointestinal upset; current smoker and high alcohol consumer ( $>$  15 standard drinks per week for males or  $>$  10 standard drinks per week for females, and less than 2 days per week alcohol free).

Participants were also selected based on their habitual dietary fibre intakes. All eligible participants completed a validated habitual dietary fibre intake food frequency questionnaire (DF-FFQ) (20) during the screening phase of the study to determine whether they were low, moderate or high dietary fibre consumers. Only participants categorised with low ( $<$  18 g/d for females and  $<$  22 g/d for males) or high ( $\geq$  25 g/d for females and  $\geq$  30 g/d for males) dietary fibre intakes were invited to participate in the study. The HDF categories were chosen to reflect the New Zealand recommended dietary fibre intake which is  $>$  25 g/d for females and  $>$  30 g/d for males (21). The LDF categories were chosen as the average dietary fibre intake in New Zealand is 17.5 g/d for females and 22.1 g/d for males which is well below the recommended dietary fibre intakes (22). To ensure that categorisation into low

and high dietary fibre groups was as accurate as possible, once recruited, participants completed four 3-day diet records. If the average dietary fibre intake from these records was outside of the pre-defined categories described above, the participants' data were excluded from the analysis.

## Interventions

The two interventions were either 16 g/d of powdered inulin-type fructan prebiotic (Beneo Orafiti® Synergy1- 50:50 inulin to fructo-oligosaccharide mix) as two 8 g/d doses for three weeks or 16 g/d of powdered placebo (Roquette Glucidex® 29 Premium- digestible maltodextrin) as two 8 g/d doses for three weeks. The two doses were consumed 30 min before breakfast and 30 min before dinner mixed into hot or cold beverages that the participants regularly consumed. A washout period of three weeks was undertaken between the two intervention phases (Figure 1). Both interventions were presented in identical packaging and the powders were similar in taste and appearance and were both low in calories (prebiotic 34 kcal/d; placebo 62 kcal/d). Participants were advised not to change their habitual dietary intake or physical activity levels, or take supplementary prebiotics or probiotic containing foods, drinks or supplements for the duration of the study.

## Study design

Participants attended an initial screening visit to the research unit where a fasted health screening blood sample was taken. Body composition was assessed using air displacement plethysmography (BodPod®; participants were fasted and wore skin tight clothing) and weight and height measurements were taken (**Figure 2**). Eligible participants were then randomised to one of two intervention orders (i.e. prebiotic then placebo or placebo then prebiotic) (Figure 1). The intervention order was randomised using a computer-based pre-generated intervention order. The researcher involved in participant recruitment and data collection, and the participants were blinded to the intervention order. Participants completed a participant questionnaire at the beginning of the study. They also completed a 3-day diet record & appetite questionnaire, fructan food frequency questionnaire (Fructan-FFQ), and had a weight measurement taken at the beginning and end of each intervention phase. A daily diary was completed by each participant during both intervention phases to assess compliance to the intervention, stool frequency and gastrointestinal symptoms. A fresh faecal sample was voided at the beginning and end of each intervention phase into a sterile container,

immediately sealed in an anaerobic bag containing an anaerobic sachet and stored at -20°C until processing (Figure 2).

#### Dietary intake analysis

Nutrient intake and food group serves were evaluated using four 3-day diet records. The 3-day diet records were completed on the three days leading up to the start of each intervention phase and the last three days of each intervention phase. The information collected in the 3-day diet records was entered into FoodWorks version 8.0 (Xyris Software Pty Ltd) by a registered Dietitian. The Australian database in FoodWorks was used (AusBrands and AusFoods 2015 data sources) so nutrient intake and food group analysis could be conducted.

Due to the absence of data regarding inulin-type fructan composition in dietary analysis software, fructan intake from diet was evaluated using a validated Fructan-FFQ (23). Four Fructan-FFQs were completed during the study, one at the beginning and one at the end of each intervention phase. The Fructan-FFQ comprised twenty-three food and drink items contributing to inulin and oligofructose intake. For each food or drink item, participants indicated the usual portion size (i.e. small, medium or large) and the number of portions consumed in the previous 7 days. Inulin and oligofructose consumed from food commodities (i.e. onion or garlic), were determined using published food composition data (24), whereas for composite foods (i.e. noodles and biscuits) the inulin and oligofructose intakes were determined first by calculating the food commodity content (i.e. wheat) of the composite food using the Food Commodity Intake Database (US Department of Agriculture and the US Environmental Protection Agency, USA) and then calculating the inulin and oligofructose amounts of each food commodity present in each composite food item, as previously performed (25). Portion sizes were estimated using standard portion size information (26).

#### Appetite rating analysis

Appetite rating was evaluated using an anchored 100 mm visual analogue scale (27). Hunger, fullness, satisfaction and how much can be consumed were assessed. Participants were instructed to mark with a cross at the point on the scale where they felt the cross best represented their appetite at the time the questionnaire was completed. Appetite ratings were assessed 30 min before and 30 min after main meals (breakfast, lunch and dinner) on the three days leading up to the start of each intervention phase and the last three days of each intervention phase.

## Bacterial DNA extraction

Faecal microbiota were measured using 16S rRNA bacterial gene sequencing and bifidobacteria concentrations were analysed using quantitative polymerase chain reaction (PCR). Bacterial DNA was extracted from the faecal samples using the MoBio PowerLyzer® Powersoil DNA® isolation kit according to the manufacturer's instructions with minor alterations. Faecal subsamples were taken from the outer region of the sample only (to reduce variability) and weighed ( $0.25 \pm 0.025$  g) into PowerLyzer® glass bead tubes. A FastPrep-24™ 5G (MP Biomedicals) was used to homogenise the samples at a speed of 5.5 m/sec for four 90 sec cycles with a 60 sec break between each cycle. The DNA was eluted in 10 mM Tris. NanoDrop 1000 spectrophotometry was used to quantify the DNA concentration.

## 16S rRNA bacterial gene sequencing

The extracted bacterial DNA was used as a template for initial PCR amplification of the V3-V4 hyper-variable region of the 16S rRNA bacterial gene using the barcoded fusion primers: 16SR\_V4 (5'-CAAGCAGAAGACGGCATACGAGAT-barcode-AGTCAGTCAGCCGGAC TACHVGGGTWTCTAAT-3') and 16SF\_V3 (5'-AATGATACGGCGACCACCGAGATCT ACAC-barcode-TATGGTAATTGGCCTACGGGAGGCAGCAG-3'), which also contain adaptors for downstream Illumina MiSeq sequencing. Each sample was amplified with a pair of unique (8 base) barcoded primers. The PCR reagents used were Invitrogen AccuPrime™ Pfx SuperMix (part number 12344-040) (17 µL), 10 µM 16SR\_V4 Primer (1 µL), 10 µM 16SF\_V3 Primer (1 µL) and Ambion nuclease-free water (catalog number: AM9932) to normalise to 5 ng/µL (1 µL). The following PCR conditions were used; a hold at 95 °C for 2 min followed by 30 cycles of 95°C for 20 sec (denaturation), 55°C for 15 sec (annealing), 72°C for 5 min (extension) finishing with a hold at 72°C for 10 min. Library clean-up utilised an Invitrogen SequelPrep Normalisation Plate Kit (Thermo Fisher). Eighteen µL of the PCR product was used in the library clean-up with an elution volume of 12 µL. A Qubit DNA high sensitivity assay was used to measure the library concentration and a Bioanalyzer DNA HS assay was used for library sizing. The libraries were pooled by equal volume. Sequencing was undertaken on an Illumina MiSeq machine, using 2 x 250 base pair (bp) read length, at the Massey Genome Service (Massey University, Palmerston North, New Zealand).

The data obtained from Illumina MiSeq sequencing were analysed using Quantitative Insights Into Microbial Ecology (QIIME) (28). Paired-end assembler for DNA sequencing



(PANDAsseq) was used to assemble the forward and reverse reads into continuous sequences ensuring at least a 50 bp overlap with a minimum of 350 bp and a maximum of 500 bp length (29). Chimera filtered sequences and reads were clustered into operational taxonomic units based on an identity threshold value of 97% using USEARCH 6.1 and UCLUST (30). Sequence alignment with the Greengenes core reference database (version 13\_5) was carried out using PyNAST (31). The RDP Naïve Bayesian classifier was used to provide taxonomic assignment (32). As there was variation in the library size (33,906 to 196,843 reads) and the potential for differing sequencing depths, which could bias the diversity metric calculations, all samples were rarefied to 33,000 reads. Sequencing data is available from the NCBI Sequence Read Archive under study SRP120250; BioProject PRJNA414683 (<https://www.ncbi.nlm.nih.gov/Traces/study/?acc=SRP120250>).

#### Quantitative PCR

In addition to 16S rRNA bacterial gene sequencing, quantitative PCR analysis of bifidobacteria was undertaken as it provides direct quantification and previous research, utilising this technique, has demonstrated that bifidobacteria response to certain dietary interventions is influenced by baseline concentrations (12–16). Therefore, bifidobacteria concentrations were determined using the LightCycler<sup>®</sup> 480 system (Roche Life Science). Standard template DNA was prepared using *Bifidobacterium bifidum* (DSM20082). *Bifidobacterium bifidum* was grown in MRS (De Man, Rogosa, Sharpe) broth (Oxoid, Adelaide, Australia) + 0.05% cysteine at 37°C for 2 days under anaerobic conditions. The culture was counted using a haemocytometer and adjusted to a final concentration of  $1 \times 10^9$  cells/mL. Bacterial DNA was extracted using the MoBio PowerLyzer<sup>®</sup> Powersoil DNA<sup>®</sup> isolation kit as described above. The following primers were used: forward (GGGTGGTAATGCCGGATG) and reverse (CCACCGTTACACCGGGAA) primers (33). Quantitative PCR was performed in triplicate with 10 µL of SyBr Green Master (Roche Life Science), 1 µL of each of the forward and reverse primers (5 µM), 7 µL of PCR grade water and 1 µL of template DNA. The conditions used for PCR amplification were initial denaturation at 95°C for 5 min followed by 40 cycles of denaturation (95°C for 1 min), annealing (66°C for 45 sec), extension (72°C for 1 min) and finished with a melt curve (95°C for 30 sec, 65°C for 1 min and 95°C continuous– 5 per °C acquisitions).

## Faecal short-chain fatty acid analysis

Short-chain fatty acids were measured by gas chromatography (GC) using a modified known method (34). While still frozen, 0.5 g to 1.0 g of faecal sample was weighed into a 15 mL Eppendorf tube; 0.01 M phosphate buffered saline containing 2-ethylbutyric acid (5.56 mM) as an internal standard was added to the faecal sample to make an aqueous faecal solution (dilution factor of 10) containing 5 mM 2-ethylbutyric acid. The samples were kept on ice and mixed to disperse faecal matter. Aqueous faecal solutions were centrifuged at 3000 x g for 10 min (4°C), 500 µL of the supernatant was transferred to a 2 mL Eppendorf tube and was acidified with 250 µL concentrated hydrochloric acid and 1000 µL diethyl ether added. Following a 10 sec vortex, to allow acids to transfer to the diethyl ether phase, the samples were centrifuged at 10,000 x g for 5 min (4°C). In a capped GC vial 100 µL of the diethyl ether phase was derivatised with 20 µL N-tert-butyldimethylsilyl-N-methyltrifluoroacetamide with 1% tert-butyldimethylchlorosilane (MTBSTFA + TBDMSCI, 99:1; Sigma-Aldrich) in a water bath at 80°C for 20 min. Once cooled, the derivatised sample was transferred to a 200 µL vial insert and recapped. To ensure complete derivatisation, the samples were left for 48 h at room temperature before analysis using GC. Standards containing 2-ethylbutyric acid (5 mM) as an internal standard were prepared for derivatisation alongside the samples.

Analysis was performed on a Shimadzu capillary gas chromatograph system (GC-2010 Plus, Tokyo, Japan) equipped with a flame ionisation detector and fitted with a Restek column (SH-Rtx-1, 30 m × 0.25 mm ID × 0.25 µm) (Shimadzu, USA). The carrier gas was helium with a total flow rate of 21.2 mL/min and pressure of 131.2 kPa. Make-up gas was nitrogen. The temperature program began at 70°C increasing to 115°C at 6°C/min, with a final increase to 300°C at 60°C/min, holding for 3 min. Flow control mode was set to linear velocity; 37.5 cm/sec. Injector temperature was 260°C and detector temperature was 310°C. Samples were injected (1 µL) with a split injection (split ratio 10:1). The GC instrument was controlled and data processed using Shimadzu GC Work Station LabSolutions Version 5.3. Data acquired provided a final sample result of µmol SCFA/g wet faeces.

## Sample size calculations

In order to detect a significant difference in responsiveness of the key phylum and genera (i.e. Actinobacteria, *Lactobacillus*, *Faecalibacterium*, *Bifidobacterium*) to the prebiotic intervention (difference of 3% in bacterial composition with a variance of 9% between and within individuals) between the LDF and HDF groups (with a power of 80% and significance

of 5%) thirty-four participants were required (35). To allow for participant withdrawal we aimed to recruit approximately forty participants.

### Statistical analysis

Mann-Whitney tests were used to determine whether there were significant differences in baseline (start of intervention phase 1) bacterial taxa, SCFA concentrations and dietary intakes (fructan intakes, nutrient intakes and food group serves) between the LDF and HDF groups. One-way repeated measures analysis of variance (ANOVA) was used to determine whether nutrient intakes changed throughout the duration of the study in the whole cohort. Differences in participant characteristics between the LDF and HDF groups were assessed using t-tests and Chi-squared tests. McNemar's tests were used to determine whether there were any differences in gastrointestinal symptom frequency during the placebo and prebiotic intervention phases in the whole cohort and the LDF and HDF groups. Differences in bacterial taxa and SCFA concentrations between the start of intervention phase 1 (baseline) and start of intervention phase 2 (after the washout period) were determined using a Mann-Whitney test. Mann-Whitney test was also used to determine whether the bacterial taxa or SCFA concentrations changed during the placebo intervention phase. Two-way repeated measures ANOVA, blocked by participant, were used to determine whether there were differences in appetite ratings, SCFA concentrations and bacterial taxa during the prebiotic and placebo intervention phases in the whole cohort and the LDF and HDF groups. Two-way repeated measures ANOVA, blocked by participant, was also used to determine whether there were differences in prebiotic driven gut microbiota response between the LDF and HDF groups. Bacterial taxa with skewed data were log transformed to help normalise the data. Only genus level bacteria with a mean relative abundance of >1% were included in the analysis unless a genus (i.e. *Faecalibacterium* and *Lactobacillus*) has been shown in the literature to be influenced by inulin-type fructan prebiotics (36). PERMANOVA analysis (adonis procedure in R package vegan) was undertaken, using the relative abundances for the unweighted UniFrac distance, to determine whether the gut microbiota community changes that occurred differed significantly between the LDF and HDF groups. Spearman's rank correlation test was used to analyse the correlation between baseline (start of intervention phase 1) bifidobacteria concentrations and change in bifidobacteria concentrations after the prebiotic intervention. Statistical analysis was carried out using Genstat version 17.1.0.14713 or R package vegan version 2.4-4. QIIME (28) was used to conduct the statistical analysis

(non-parametric two-sample t-test) to compare baseline (start of intervention phase 1) alpha diversity between the dietary fibre groups and the change in alpha diversity in the LDF and HDF groups after the prebiotic intervention.

## RESULTS

### Participants

Of the forty-four eligible participants who provided informed consent to participate in the study, four did not complete the study as they either experienced severe gastrointestinal symptoms (i.e. disabling abdominal pain, cramps and bloating) due to the prebiotic (n = 2) or were prescribed antibiotics at the beginning of the study (n = 1). One participant was also prescribed antibiotics at the end of the study (during the placebo intervention phase), however, the data collected during the prebiotic intervention phase were still able to be used. Forty participants completed the study, however, the data from seven participants were excluded as the participants were either assessed as being moderate dietary fibre consumers, based on the data collected from the four 3-day diet records (n = 6), or were found to have consumed supplementary prebiotics and probiotic containing foods and drinks during the study (n = 1). The data collected from thirty-four participants were used for the prebiotic intervention analysis and the data collected from thirty-three participants were used for the placebo intervention analysis (Figure 1).

### Baseline dietary intake and participant characteristic differences

Categorisation into different dietary fibre intake groups was successful as dietary fibre intakes were significantly different ( $p < 0.001$ ) between the LDF (n = 14; 18.0 g/d) and HDF (n = 20; 38.6 g/d) groups. There were several additional significant differences ( $p < 0.05$ ) in baseline nutrient intakes between the LDF and HDF groups. HDF consumers had higher energy, total fat, polyunsaturated fat, monounsaturated fat, carbohydrate and dietary fibre per 1000 kJ compared to the LDF group. There were, however, no differences in fructan intake between the two groups (**Table 1**). Energy from fat (%) and energy from protein (%) were not significantly different, however, energy from fibre (%) was significantly different between dietary fibre groups ( $p < 0.001$ ). Therefore, the only macronutrient that continued to be significantly different between dietary fibre groups after energy intakes were controlled for was dietary fibre (Table 1). The LDF group had a lower intake of fruits ( $p = 0.009$ ), vegetables ( $p < 0.001$ ) (dark green [ $p = 0.007$ ] and red orange vegetables [ $p = 0.039$ ]), protein

foods ( $p = 0.036$ ) and nuts and seeds ( $p = 0.001$ ) compared to the HDF group (**Figure 3**). Dietary intakes did not change throughout the duration of the study (**Supplemental Table 1**). Despite similar age, sex and BMI, there were significant differences in body composition between the two dietary fibre groups. The LDF group had a significantly lower fat free mass ( $p = 0.021$ ) and significantly higher fat mass ( $p = 0.021$ ) compared to the HDF group (**Table 2**).

#### Baseline SCFA concentration and microbiota differences

Of the 138 faecal samples analysed, a total of 12,420,607 high quality 16S rRNA bacterial gene sequence reads were generated. The average number of sequence reads generated per faecal sample was 90,004 (33,906 to 196,843 reads per sample).

There were no significant baseline differences in SCFA concentrations (**Table 3**) or any of the alpha diversity indices measured (Observed species, Shannon, Chao and PD\_whole tree) (**Supplemental Table 2**) between the LDF and HDF groups. At baseline the relative abundance of an unknown genus of *Lachnospiraceae* (other) was significantly higher in the LDF group compared to the HDF group ( $p = 0.043$ ). The LDF group also had a trend towards a higher relative abundance of *Bifidobacterium* compared to the HDF group; however, statistical significance was not reached ( $p = 0.066$ ) (**Table 3**).

#### Gastrointestinal symptoms

In the whole cohort, the frequency of mild and severe gastrointestinal symptoms were statistically similar ( $p > 0.05$ ) during the placebo and prebiotic intervention phases. There was, however, a significantly higher frequency of moderate symptoms ( $p = 0.013$ ), particularly moderate flatulence ( $p = 0.012$ ), experienced during the prebiotic compared to the placebo intervention phase (**Supplemental Table 3**).

After categorisation into dietary fibre intake groups, the HDF group also experienced an increased frequency of moderate symptoms ( $p = 0.004$ ), particularly moderate flatulence ( $p = 0.016$ ), during the prebiotic compared to the placebo intervention phase. There were no significant differences in gastrointestinal symptom frequency between the placebo and prebiotic intervention phases in the LDF group (**Supplemental Table 3**).

### Prebiotic driven changes in appetite ratings

In the whole cohort, there were no significant differences in appetite ratings before or after breakfast, lunch or dinner during the prebiotic intervention phase (**Supplemental Table 4**).

After categorising participants based on their dietary fibre intakes, appetite ratings did not significantly change before or after breakfast, lunch or dinner during the prebiotic intervention phase in the LDF group (**Supplemental Table 5**). There were, however, a number of significant changes in appetite ratings during the prebiotic intervention phase in the HDF group. The prebiotic intervention led to a significant reduction in satisfaction before lunch ( $p = 0.042$ ) and in hunger after dinner ( $p = 0.006$ ), and a significant increase in fullness ( $p = 0.002$ ) and satisfaction after lunch ( $p = 0.044$ ) (**Supplemental Table 6**).

### Prebiotic driven changes in SCFA concentrations and microbiota

Gut microbiota composition and SCFA concentrations after the washout period were not significantly different from baseline (data not presented). There were also no significant changes in gut microbiota composition or SCFA concentrations during the placebo intervention phase (data not presented).

In the whole cohort, there were no significant changes in SCFA concentrations due to the prebiotic intervention (**Table 4**). There were, however, a number of prebiotic driven changes in bacterial taxa. At a phylum level, Actinobacteria relative abundance significantly increased ( $p < 0.001$ ) and Firmicutes relative abundance significantly decreased ( $p = 0.007$ ). There was also a trend towards a reduction in Proteobacteria relative abundance ( $p = 0.070$ ) during the prebiotic intervention phase (Table 4). At a genus level, there was a prebiotic driven increase in the relative abundance of *Bifidobacterium* ( $p < 0.001$ ) and a reduction in *Coprococcus* ( $p = 0.016$ ), *Dorea* ( $p = 0.029$ ), *Ruminococcus* (*Lachnospiraceae* family) ( $p = 0.007$ ) and *Oscillospira* relative abundance ( $p = 0.031$ ). There was also a trend towards an increase in *Faecalibacterium* relative abundance ( $p = 0.088$ ) during the prebiotic intervention phase (Table 4).

After categorisation into dietary fibre intake groups, there were no significant prebiotic driven changes in SCFA concentrations in the LDF (**Supplemental Table 7**) or HDF groups (**Supplemental Table 8**) which was consistent with the whole cohort analysis (Table 1). At a phylum level, both dietary fibre groups had a significant increase in Actinobacteria relative abundance (LDF  $p = 0.007$  and HDF  $p = <0.001$ ); however, the reduction in Firmicutes relative abundance was only significant in the HDF group (LDF  $p =$

0.127 and HDF  $p = 0.027$ ) (**Figure 4** + Supplemental Tables 7 & 8). At a genus level, the only significant change that occurred during the prebiotic intervention in the LDF group was an increase in the relative abundance of *Bifidobacterium* ( $p = 0.001$ ) (**Figure 5** + Supplemental Table 7). The prebiotic intervention did, however, lead to a number of significant changes in the HDF group including a significant increase in *Bifidobacterium* ( $p < 0.001$ ) and *Faecalibacterium* relative abundance ( $p = 0.010$ ), and a significant reduction in *Coprococcus* ( $p = 0.010$ ), *Dorea* ( $p = 0.043$ ) and *Ruminococcus* (*Lachnospiraceae* family) relative abundance ( $p = 0.032$ ) (Figure 5 + Supplemental Table 8). There was a trend towards a reduction in Shannon index ( $p = 0.060$ ) in the HDF group and an increased in Chao index ( $p = 0.060$ ) in the LDF group during the prebiotic intervention (Supplemental Table 2).

The unweighted UniFrac distance principal co-ordinate analysis biplots ( $\beta$  diversity) demonstrate that there were large inter-individual variability in whole community microbiota responses to the inulin-type fructan prebiotic (**Figure 6**). For example, participant 32 (LDF) and 28 (HDF) harboured gut microbiota communities that were more responsive to the inulin-type fructan prebiotic as their before (black dot) and after (grey dot) prebiotic intervention samples are a distance away from each other. However, participant 19 (LDF) and 03 (HDF) before (black dot) and after (grey dot) prebiotic intervention samples cluster together suggesting that their gut microbiota communities were less responsive to the inulin-type fructan prebiotic. There was, however, no significant difference in how the gut microbiota communities responded to the inulin-type fructan between the LDF and HDF groups ( $p = 0.997$ ).

The between dietary fibre group comparison demonstrated that there were no differences in phylum level gut microbiota response to the prebiotic between the LDF and HDF groups (**Table 5**). There were also no differences in prebiotic driven SCFA production between the LDF and HDF groups (Table 5). The gut microbiota did, however, respond differently between the LDF and HDF groups at a genus level for *Lactobacillus*, an unknown genus of *Ruminococcaceae* and *Faecalibacterium* (**Figure 7**). There was minimal change in *Lactobacillus* relative abundance due to the prebiotic in the HDF group; however, *Lactobacillus* increased from 0.6% to 3.0% in the LDF group ( $p = 0.025$ ). The relative abundance of an unknown genus of *Ruminococcaceae* increased in the HDF group but decreased in the LDF group ( $p = 0.018$ ). The relative abundance of *Faecalibacterium* increased more markedly in the HDF group than the LDF group ( $p = 0.009$ ) (Table 5).



## Correlation between baseline bifidobacteria concentrations and change in bifidobacteria

The quantitative PCR data was used to determine whether there was a correlation between baseline bifidobacteria concentrations and change in bifidobacteria concentrations due to the prebiotic intervention. A significant correlation was demonstrated for both the LDF ( $p = 0.017$ ) and HDF ( $p = 0.004$ ) groups. The strength of the correlation was similar between the dietary fibre groups (**Figure 8**).

## DISCUSSION

In the present study, the inulin-type fructan prebiotic led to several microbial changes in the whole cohort including an increase in *Bifidobacterium* and a decrease in *Coprococcus*, *Dorea*, *Ruminococcus* (*Lachnospiraceae* family) and *Oscillospira* relative abundances. There was also a trend towards an increase in *Faecalibacterium* relative abundance. Previous inulin-type fructan prebiotic intervention studies have demonstrated similar results with increases in *Bifidobacterium* and/or *Faecalibacterium* being reported in a number of studies (14,36–39). Short-chain fatty acid concentrations did not differ after the prebiotic intervention. *In vitro* studies have shown that inulin-type fructan prebiotics lead to an enhanced production of butyrate (40,41); however, this result is often not replicated in human prebiotic intervention studies (35,38,39). This is not overly surprising as over 95% of the SCFAs produced in the human colon are used by the microbiota that reside within the gut, are rapidly utilised by colonocytes and are absorbed into the hosts systemic circulation (42,43). Additionally, there were no significant changes in appetite ratings during the prebiotic intervention phase in the whole cohort.

Interestingly, categorisation into LDF and HDF groups led to a number of distinctions in gut microbiota response within each dietary fibre group. In the LDF group, the only significant genus level microbiota change elicited by the inulin-type fructan prebiotic was an increase in *Bifidobacterium* relative abundance. In the HDF group, the inulin-type fructan prebiotic led to a significant increase in the relative abundance of *Bifidobacterium* and *Faecalibacterium* and a significant reduction in *Coprococcus*, *Dorea* and *Ruminococcus* (*Lachnospiraceae* family). The LDF group appeared to harbour a gut microbiota community that were more resilient to change and, therefore, less responsive to the inulin-type fructan prebiotic than the HDF group. A study conducted by Eid and co-authors (17) demonstrated that individuals with an average dietary fibre intake of 18 g/d hosted gut microbiota that were more stable to a palm date intervention. In their study individuals with an average dietary



fibre intake of 18 g/d were classified as HDF, whereas, in the present study LDF consumers had an average dietary fibre intake of 18 g/d. A recent study, which used germ-free (gnotobiotic) mice colonised with human gut microbiota from donors with two varying dietary patterns (typical American style dietary pattern [AMER] or a plant-rich, calorie-restricted diet with optimal nutrient composition [CRON]), demonstrated that mice inoculated with AMER microbiota were less responsive to the CRON type diet when compared to mice inoculated with CRON microbiota (18). A recent *in vitro* batch fermentation study demonstrated that donors with healthier dietary patterns harboured gut microbiota that were better equipped at utilising fermentable carbohydrates found in grains compared to donors with less healthy dietary patterns (44). Therefore, the HDF group in the present study may have gut microbiota consortia that are metabolically more capable of utilising high amounts of fermentable substrates as their habitual diet is already high in these substrates.

The between dietary fibre group comparison demonstrated that there were several bacterial genera that responded in a distinctive manner between the LDF and HDF groups. The inulin-type fructan prebiotic led to an increase in the relative abundance of *Faecalibacterium* and an unknown genus of *Ruminococcaceae*, and minimal change in *Lactobacillus* relative abundance in the HDF group. In the LDF group, the inulin-type fructan prebiotic led to an increase in the relative abundance of *Lactobacillus*, minimal change in *Faecalibacterium* relative abundance and a reduction in the relative abundance of an unknown genus of *Ruminococcaceae*. It is likely that the whole cohort bacterial taxa results may have changed if the number of LDF and HDF participants recruited were different. In the present study, the data from more HDF than LDF consumers (20 and 14 participants; respectively) were used in the analysis. If the proportion of recruited HDF to LDF participants differed from the present study, then this could have had implications on the whole cohort results. For example, if more LDF than HDF consumers were recruited the prebiotic may have led to a significant increase in the relative abundance of *Lactobacillus*.

Host-specific responses, such as appetite rating changes and gastrointestinal symptoms, were significantly influenced by the inulin-type fructan prebiotic in the HDF group only. The HDF group reported a significantly higher frequency of moderate flatulence, an increase in fullness and satisfaction after lunch and a reduction in hunger after dinner due to the inulin-type fructan prebiotic. One of the key differences in gut microbiota response to the prebiotic intervention between dietary fibre groups was the significant increase in *Faecalibacterium* relative abundance observed only in the HDF group. Butyrate and CO<sub>2</sub> are

primary metabolic by-products of indigestible substrate fermentation by *Faecalibacterium* in the colon (45). A primary component of flatus is CO<sub>2</sub> gas (46) and butyrate has been shown to be involved in regulating appetite-associated gut hormones (47). Therefore, it is plausible that the significant change in appetite ratings and the increased frequency of moderate flatulence experienced by the HDF group after the prebiotic intervention may be associated with the increased abundance of *Faecalibacterium*. Nevertheless, further investigation is required to demonstrate whether a link between *Faecalibacterium* and host response in healthy individuals exists.

Consideration towards inter-individual variability in gut microbiota responsiveness will be particularly important when researching the prebiotic potential of a given dietary intervention for the first time. If a greater proportion of participants with less responsive gut microbiota communities are recruited then it may appear that the dietary intervention does not have an influence on the gut microbiota which may not be representative of the true prebiotic efficacy of the dietary intervention for all participants. Gaining additional insight into the factors which influence gut microbiota responsiveness, so they can be controlled for more effectively, will help determine the true prebiotic efficacy of a dietary intervention and provide better consistency of results between studies.

Quantitative PCR data were utilised to investigate whether a correlation between baseline bifidobacteria concentrations and prebiotic driven change in bifidobacteria concentrations exists in our study cohort. We also aimed to determine whether the strength of the correlation differed between the LDF and HDF groups. A significant correlation did exist between baseline bifidobacteria concentration and prebiotic driven change in bifidobacteria concentration with lower baseline bifidobacteria concentrations being correlated with a more pronounced bifidogenic response. The strength of the correlation did not differ between the LDF and HDF groups, suggesting that habitual dietary fibre intakes do not have an influence on the correlation. The majority of previous studies are in agreement with our results as they have also observed that lower baseline bifidobacteria concentrations lead to a more pronounced increase in bifidobacteria in response to a dietary intervention (12–16).

There are a number of strengths of this study including the robust study design: a randomised, double-blind, placebo-controlled, cross-over human intervention study. This is also the first study to recruit participants based on pre-defined habitual dietary fibre intake categories to demonstrate what influence habitual dietary fibre intake has on gut microbiota responsiveness. Another strength of this study is the utilisation of next-generation sequencing technology which allowed for the characterisation of the whole microbial community rather

than focusing on changes that occur in only select bacterial taxa. A limitation of the present study is that the interpretation of our results is limited to inulin-type fructan prebiotic interventions, in particular, a mixed inulin:fructo-oligosaccharide prebiotic. Numerous other fermentable carbohydrates have a proven prebiotic effect, including galacto-oligosaccharides (GOS), however, the impact of habitual dietary fibre intake on the responsiveness of the gut microbiota to other prebiotics has not been published previously, nor was it investigated here. The microbially-derived enzymes, fructanase (responsible for fructan fermentation) and  $\beta$ -Galactosidase (responsible for GOS fermentation) (48) are encoded on various bacteria and therefore the diet-dependent effects on prebiotic specificity may be different for different prebiotics (49). The influence habitual dietary fibre intake has on the responsiveness of the gut microbiota to other dietary interventions, such as GOS, calorie restriction, increased resistant starch and high wholegrains diets, will need to be researched in the future.

In conclusion, it is difficult to predict how the gut microbiota will respond to a dietary intervention. Gaining a better understanding of the factors implicated in inter-individual variability in gut microbiota responsiveness may help improve dietary intervention success and subsequently enhance human health outcomes. In this study, we identified that individuals with HDF intakes have a greater gut microbiota response to an inulin-type fructan prebiotic. These individuals also experienced greater benefits in appetite but reported more gastrointestinal symptoms. Future studies aiming to modulate the gut microbiota using an inulin-type fructan prebiotic should take habitual dietary fibre intake into account either when recruiting participants or during data analysis to help minimise the influence inter-individual variability in gut microbiota responsiveness has on study outcomes.

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## AUTHORSHIP

The authors contributions were as follows: GH was involved in the conception, study design, participant recruit, running of the study, sample processing, data collection, analysis and interpretation, as well as the writing of the manuscript. LB, CB, RM and JC were involved in the conception, study design and data interpretation. KW was involved in the study design and provided expertise relating to the Fructan-FFQ. All authors contributed to the development and approval of the manuscript. The authors report no conflict of interest.

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## CONFLICT OF INTEREST

The authors report no conflict of interest.

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## FIGURE LEGENDS

Figure 1- Consolidated Standards of Reporting Trials (CONSORT) flow diagram.

Figure 2- Participant flow through the study including measurements, questionnaires and samples taken at each research unit visit. IP: intervention phase, Fructan-FFQ: fructan food frequency questionnaire, BodPod: air displacement plethysmography.

Figure 3- Baseline differences in the average number of food group serves consumed per day (as assessed using four 3-day diet records) between the low and high dietary fibre groups. Changes that are significantly different ( $p < 0.05$ ) between dietary fibre groups are indicated with an asterisks (\*) as analysed by a Mann-Whitney test.

Figure 4- Mean phylum level relative abundance (%) before and after the placebo and prebiotic intervention phases for the low (LDF) and high habitual dietary fibre (HDF) groups. Values that are significantly different from the placebo intervention and prebiotic before intervention are indicated with an asterisk; \* $p < 0.05$ , \*\* $p < 0.01$ .

Figure 5- Mean genus level relative abundance (%) before and after the placebo and prebiotic intervention phases for the low (LDF) and high habitual dietary fibre (HDF) groups. After intervention values that are significantly different from the placebo intervention and prebiotic before intervention (or in the case of *Dialister* significantly different from those of the

prebiotic intervention and placebo before intervention) are indicated with an asterisk; \* $p < 0.05$ , \*\* $p < 0.01$ .

Figure 6- Principal co-ordinate analysis biplots (unweighted UniFrac distances) illustrating the between sample differences in bacterial taxa ( $\beta$ -diversity) before (black dots) and after the prebiotic intervention (grey dots) for the low and high habitual dietary fibre groups. Participant IDs are shown on the biplot (i.e. 03). The further apart a participants' samples are from each other the greater the whole community microbiota response was to the inulin-type fructan prebiotic. The white shaded spheres represent the 10 most abundant bacterial taxa. The spheres that cluster in the middle of the graphs which are not labelled include the following bacterial taxa: *Coprococcus*; *Bacteroides*; *Ruminococcus*; *Collinsella*; *Lachnospiraceae*, unknown genus; *Blautia* and *Ruminococcaceae*, unknown genus. The position and size of the sphere indicates the bacterial taxa that are the most influential in driving the separation of the samples.

Figure 7- Mean genus level changes after the prebiotic intervention between the low and high dietary fibre groups. A significant change ( $p < 0.05$ ) is indicated with an asterisk (\*) as analysed by a two-way repeated-measures ANOVA (blocked by participant) and least significant difference test.

Figure 8- The correlation between baseline bifidobacteria concentrations (Before [log]) and change in bifidobacteria concentrations (After over before [log]) during the prebiotic intervention between the low and high dietary fibre groups. Bifidobacteria concentrations were determined using quantitative PCR. P values  $< 0.05$  are considered significant as analysed by a Pearson's rank correlation test.

## TABLES

**TABLE 1**

Baseline dietary intake differences between the low and high dietary fibre groups<sup>1</sup>

Dietary intake	Low dietary fibre (n=14)		High dietary fibre (n=20)		P value
	Mean	SD	Mean	SD	
Energy (kJ/d)	7161.1	2285.2	10013.9	2769.8	<b>0.002</b>
Protein (g/d)	83.1	28.5	112.7	45.5	0.066
Total fat (g/d)	67.8	26.0	95.9	29.7	<b>0.012</b>
Saturated fat (g/d)	26.5	13.3	33.3	14.6	0.259
Polyunsaturated fat (g/d)	10.8	4.2	16.3	5.9	<b>0.005</b>
Monounsaturated fat (g/d)	24.6	8.9	38.0	10.8	<b>0.001</b>
Carbohydrate (g/d)	178.0	83.0	241.3	84.0	<b>0.015</b>
Sugars (g/d)	77.8	47.0	106.2	40.4	0.051
Starch (g/d)	99.2	39.5	132.0	59.9	0.104
Dietary fibre (g/d)	18.0	3.4	38.6	13.0	<b>&lt;0.001</b>
Dietary fibre (g/d) per 1000kJ	2.7	0.8	3.9	1.0	<b>&lt;0.001</b>
Total Inulin (g/d)	3.1	1.3	2.9	1.1	0.796
Total oligofructose (g/d)	3.0	1.2	2.8	1.0	0.769
Water (g/d)	2048.2	746.3	2781.4	1428.1	0.104
Alcohol (g/d)	3.1	6.8	4.2	12.3	0.565
Energy from protein (%)	20.6	8.0	19.1	4.5	0.877
Energy from fat (%)	34.4	5.2	35.9	8.5	0.986
Energy from saturated fat (%)	13.1	3.9	12.3	4.5	0.457
Energy from carbohydrate (%)	40.6	10.4	39.3	7.4	0.823
Energy from alcohol (%)	1.2	2.6	1.0	2.7	0.601
Energy from fibre (%)	2.2	0.7	3.1	0.8	<b>&lt;0.001</b>

<sup>1</sup> Mann-Whitney test. Significant results (p < 0.05) are in **bold**. SD: standard deviation

**TABLE 2**

Participant characteristic comparison between the low and high dietary fibre groups<sup>1</sup>

	Low dietary fibre (n=14)	High dietary fibre (n=20)	P value
Age (years)	37.7 ± 10.6	37.2 ± 14.4	0.902
BMI (kg/m <sup>2</sup> )	24.3 ± 2.7	22.5 ± 2.8	0.061
Male : Female	6 : 8	7 : 13	0.643
Fat mass (%)	27.5 ± 7.5	20.3 ± 9.0	<b>0.021</b>
Fat free mass (%)	72.6 ± 7.5	79.7 ± 9.0	<b>0.021</b>
Ethnicity (no.)			0.938
NZ European	6	9	
Maori	1	2	
Other	7	9	
Skip meals (Yes : No)	6 : 8	5 : 15	0.273
Snack consumed per day (no.)	2.0 ± 0.9	2.4 ± 0.6	0.091
Activity level <sup>^</sup>	5.1 ± 0.9	5.6 ± 1.2	0.183
Stools passed per week (no.)	6.7 ± 3.6	8.6 ± 3.8	0.155

<sup>1</sup> Chi-squared test and unpaired t-test. Significant results (p < 0.05) are in **bold**. Values are means ± standard deviations. <sup>^</sup> Activity level of 5 is seated work with some moving around and strenuous leisure activity

**TABLE 3**Baseline short-chain fatty acid concentrations and bacterial taxa in the low and high dietary fibre groups<sup>1</sup>

	Low dietary fibre (n=14)		High dietary fibre (n=20)		P value
	Mean	SD	Mean	SD	
<b>Short-chain fatty acids (μmol/g)</b>					
Acetate	28.97	18.23	33.32	19.74	0.592
Butyrate	7.77	5.10	9.08	5.80	0.545
Propionate	9.99	6.31	10.05	8.90	0.666
Sum of short-chain fatty acids	51.02	29.00	56.57	33.12	0.877
<b>Phylum (% relative abundance)</b>					
Actinobacteria	13.98	9.42	8.87	5.97	0.104
Bacteroidetes	11.31	8.94	16.82	11.44	0.169
Firmicutes	72.82	8.79	72.12	12.15	0.931
Proteobacteria	0.65	0.93	0.47	0.47	0.823
Verrucomicrobia	0.33	0.58	0.26	0.33	0.304
<b>Genus (% relative abundance)</b>					
<i>Bifidobacterium</i>	9.81	7.78	4.51^	4.10	0.066
<i>Collinsella</i>	2.95	3.09	3.15	2.69	0.616
<i>Bacteroides</i>	6.77	5.01	6.81	3.66	0.931
<i>Prevotella</i>	2.94	4.89	6.79	11.03	0.666
<i>Lactobacillus</i>	0.59	1.29	0.03	0.06	0.609
<i>Lachnospiraceae</i> , other, unknown genus	2.38	1.46	1.50*	0.78	0.043
<i>Lachnospiraceae</i> , unknown genus	11.85	7.20	13.04	5.58	0.377
<i>Blautia</i>	10.42	5.57	9.53	4.36	0.569
<i>Coprococcus</i>	3.83	2.13	4.97	2.74	0.204
<i>Dorea</i>	2.01	1.22	1.57	0.71	0.341
<i>Ruminococcus (Lachnospiraceae)</i>	2.55	1.99	1.81	1.20	0.306
<i>Ruminococcaceae</i> , unknown genus	15.95	5.08	14.74	3.12	0.569
<i>Faecalibacterium</i>	0.39	0.29	0.42	0.18	0.500
<i>Oscillospira</i>	1.21	0.65	1.00	0.42	0.478
<i>Ruminococcus (Ruminococcaceae)</i>	5.80	4.26	5.27	3.47	0.849
<i>Dialister</i>	1.00	1.65	1.12	1.81	0.568

<sup>1</sup> Mann-Whitney test. Mean values are significantly different from the low dietary fibre group. \*p < 0.05, <sup>^</sup>trend towards significance (p < 0.1). SD: standard deviation

**TABLE 4**Short-chain fatty acid concentration and bacterial taxa changes during the placebo and prebiotic intervention phases in the whole cohort<sup>1</sup>

	Placebo (n = 33)				Prebiotic (n = 34)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Short-chain fatty acids (μmol/g)</b>								
Acetate	31.78	17.80	33.80	18.97	31.53	18.97	39.50	20.96
Butyrate	9.75	6.12	9.44	5.62	8.54	5.48	10.16	5.62
Propionate	10.09	6.19	11.63	7.78	10.03	7.83	11.94	7.47
Sum of short-chain fatty acids	55.52	28.69	59.48	32.28	54.28	31.16	65.51	32.48
<b>Phylum (% relative abundance)</b>								
Actinobacteria	10.88	6.43	10.84	7.24	10.98	7.87	19.95**	10.20
Bacteroidetes	14.30	12.09	13.09	8.39	14.55	10.70	12.46	8.33
Firmicutes	72.90	11.31	73.85	11.02	72.41	10.75	65.71**	11.03
Proteobacteria	0.43	0.40	0.51	0.61	0.54	0.69	0.36^	0.42
Verrucomicrobia	0.33	0.66	0.38	0.85	0.29	0.45	0.17	0.33
<b>Genus (% relative abundance)</b>								
<i>Bifidobacterium</i>	6.56	5.21	6.50	5.88	6.69	6.37	15.07**	8.54
<i>Collinsella</i>	3.36	2.52	3.15	2.80	3.07	2.82	3.81	2.79
<i>Bacteroides</i>	6.49	3.81	6.45	4.31	6.80	4.19	5.86	3.40
<i>Prevotella</i>	5.36	12.16	3.66	5.98	5.20	9.12	4.85	7.98
<i>Lactobacillus</i>	0.24	0.92	0.44	1.96	0.26	0.86	1.26	3.83
<i>Lachnospiraceae</i> , other, unknown genus	2.07	1.11	1.91	1.24	1.86	1.18	1.55	0.62
<i>Lachnospiraceae</i> , unknown genus	13.27	5.79	13.43	5.62	12.55	6.22	14.74	6.30
<i>Blautia</i>	10.78	5.81	9.45	4.43	9.90	4.83	7.67	3.88
<i>Coprococcus</i>	3.80	1.83	4.16	2.20	4.50	2.54	3.55*	1.65
<i>Dorea</i>	1.65	0.86	1.61	0.86	1.75	0.96	1.20*	0.66
<i>Ruminococcus (Lachnospiraceae)</i>	1.85	1.66	1.95	1.64	2.11	1.59	1.15**	1.04
<i>Ruminococcaceae</i> , unknown genus	16.33	4.82	16.86	4.29	15.24	4.01	14.50	4.12
<i>Faecalibacterium</i>	0.47	0.32	0.53	0.30	0.41	0.22	0.61^	0.32
<i>Oscillospira</i>	1.10	0.67	1.11	0.70	1.08	0.53	0.78*	0.46
<i>Ruminococcus (Ruminococcaceae)</i>	5.60	3.73	5.52	4.00	5.49	3.76	4.40	3.32
<i>Dialister</i>	0.77	1.15	1.00^	1.56	1.07	1.72	0.94	1.59

<sup>1</sup> Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean values are significantly different from those of the placebo intervention and prebiotic before intervention or in the case of *Dialister* different from those of the prebiotic intervention and placebo before intervention; \*p < 0.05, \*\*p < 0.01, ^trend towards significance (p < 0.1). SD: standard deviation

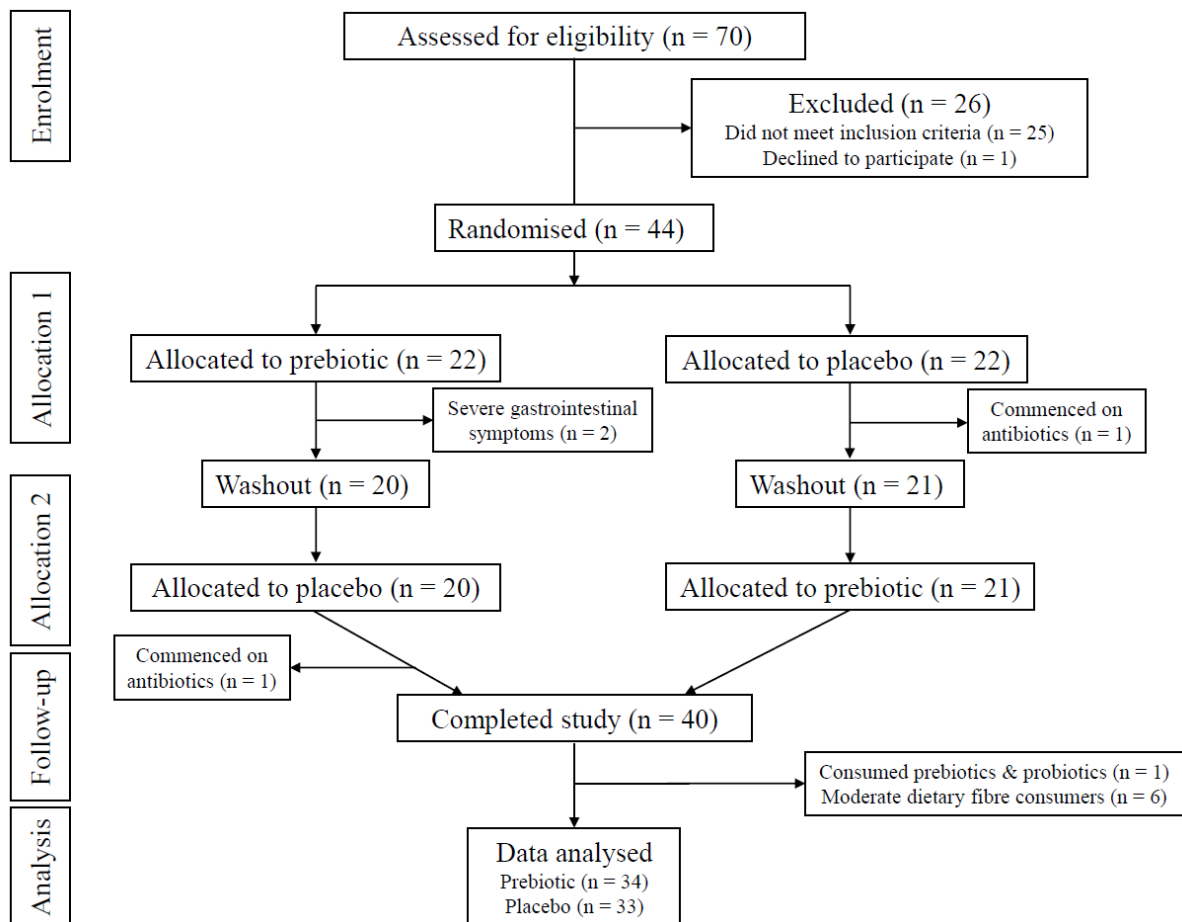
TABLE 5

Short-chain fatty acid concentrations and bacterial taxa before and after the prebiotic intervention in low and high dietary fibre groups<sup>1</sup>

	Low dietary fibre (n=14)					High dietary fibre (n=20)					P value
	Before intervention		After intervention		Change	Before intervention		After intervention		Change	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		
<b>Short-chain fatty acids (μmol/g)</b>											
Acetate	29	18.2	34.3	22.3	5.3	33.3	19.7	43.2	19.7	9.9	0.534
Butyrate	7.8	5.1	8.3	5.3	0.5	9.1	5.8	11.5	5.6	2.4	0.375
Propionate	10	6.3	10.9	7.1	0.9	10.1	8.9	12.7	7.8	2.6	0.424
Sum of short chain fatty acids	51	29	57.6	34	6.6	56.6	33.1	71.1	31.1	14.5	0.475
<b>Phylum (% relative abundance)</b>											
Actinobacteria	14	9.4	23.2	9.6	9.2	8.9	6	17.7	10.2	8.8	0.907
Bacteroidetes	11.3	8.9	9.6	5.4	-1.7	16.8	11.4	14.5	9.5	-2.3	0.829
Firmicutes	72.8	8.8	66	9.7	-6.9	72.1	12.2	65.5	12.1	-6.6	0.933
Proteobacteria	0.7	0.9	0.3	0.4	-0.4	0.5	0.5	0.4	0.5	-0.1	0.188
Verrucomicrobia	0.3	0.6	0.2	0.5	-0.1	0.3	0.3	0.2	0.2	-0.1	0.947
<b>Genus (% relative abundance)</b>											
<i>Bifidobacterium</i>	9.8	7.8	18	7.9	8.2	4.5	4.1	13	8.6	8.5	0.9
<i>Collinsella</i>	3	3.1	3.9	3.2	1	3.2	2.7	3.7	2.5	0.6	0.681
<i>Bacteroides</i>	6.8	5	5.7	3.9	-1	6.8	3.7	6	3.1	-0.9	0.909
<i>Prevotella</i>	2.9	4.9	2.5	3.6	-0.5	6.8	11	6.5	9.7	-0.3	0.898
<i>Lactobacillus</i>	0.6	1.3	3	5.6	2.4	0	0.1	0.1	0.2	0.1	<b>0.025</b>
<i>Lachnospiraceae</i> , other, unknown genus	2.4	1.5	1.8	0.6	-0.5	1.5	0.8	1.4	0.5	-0.2	0.261
<i>Lachnospiraceae</i> , unknown genus	11.9	7.2	13.2	7.4	1.4	13	5.6	15.8	5.4	2.8	0.522
<i>Blautia</i>	10.4	5.6	8.1	4.1	-2.3	9.5	4.4	7.4	3.8	-2.2	0.917
<i>Coprococcus</i>	3.8	2.1	3.1	1.9	-0.8	5	2.7	3.9	1.4	-1.1	0.65
<i>Dorea</i>	2	1.2	1.2	0.5	-0.8	1.6	0.7	1.2	0.8	-0.4	0.253
<i>Ruminococcus (Lachnospiraceae)</i>	2.6	2	1.2	1.3	-1.3	1.8	1.2	1.1	0.8	-0.7	0.249
<i>Ruminococcaceae</i> , unknown genus	16	5.1	13.1	4.8	-2.9	14.7	3.1	15.5	3.3	0.8	<b>0.018</b>
<i>Faecalibacterium</i>	0.4	0.3	0.5	0.3	0.1	0.4	0.2	0.7	0.3	0.3	<b>0.009</b>
<i>Oscillospira</i>	1.2	0.7	0.9	0.5	-0.3	1	0.4	0.7	0.5	-0.3	0.986
<i>Ruminococcus (Ruminococcaceae)</i>	5.8	4.3	5	4	-0.8	5.3	3.5	3.9	2.8	-1.3	0.617
<i>Dialister</i>	1	1.7	1.1	2.1	0.1	1.1	1.8	0.8	1.1	-0.3	0.356

<sup>1</sup> Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. The changes in bacterial relative abundance that were significantly different between the low and high dietary fibre groups are in **bold** (p < 0.05). SD: standard deviation.

Figure 1



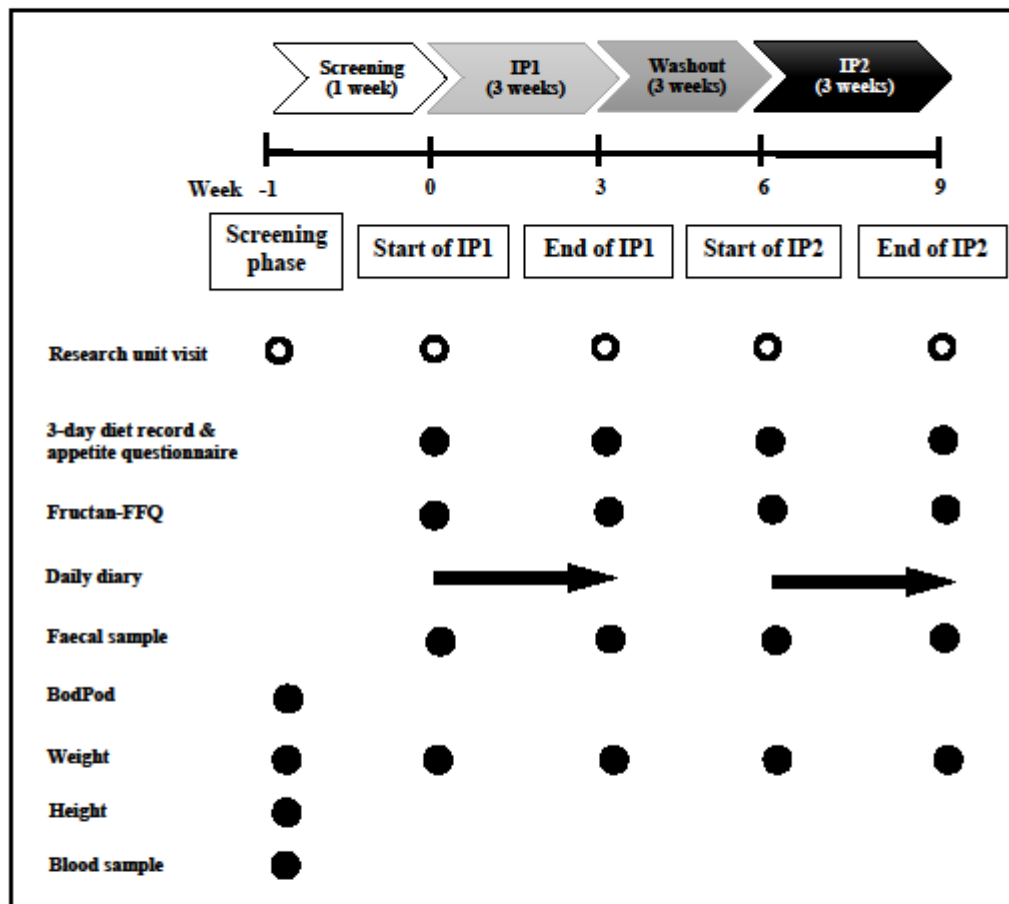


Figure 2



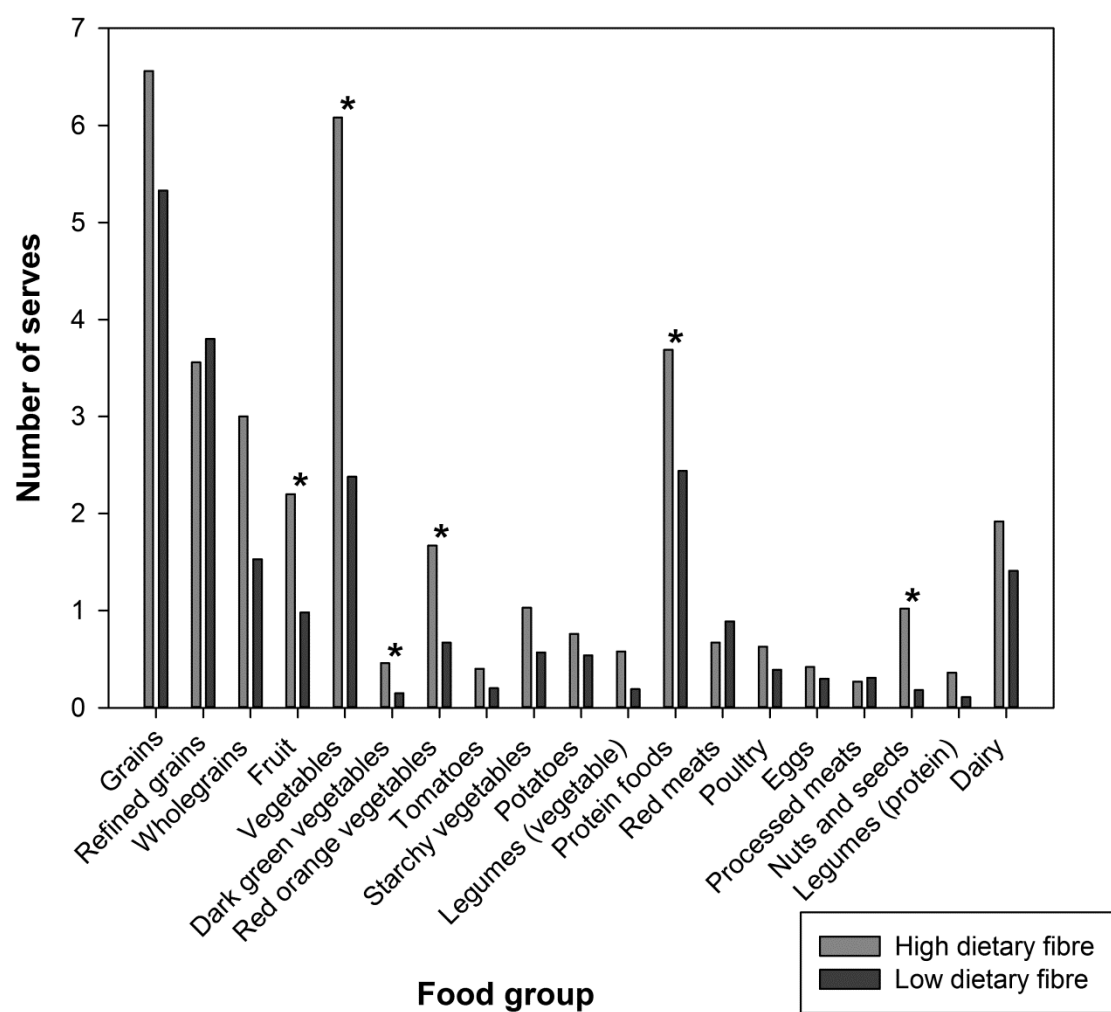


Figure 3

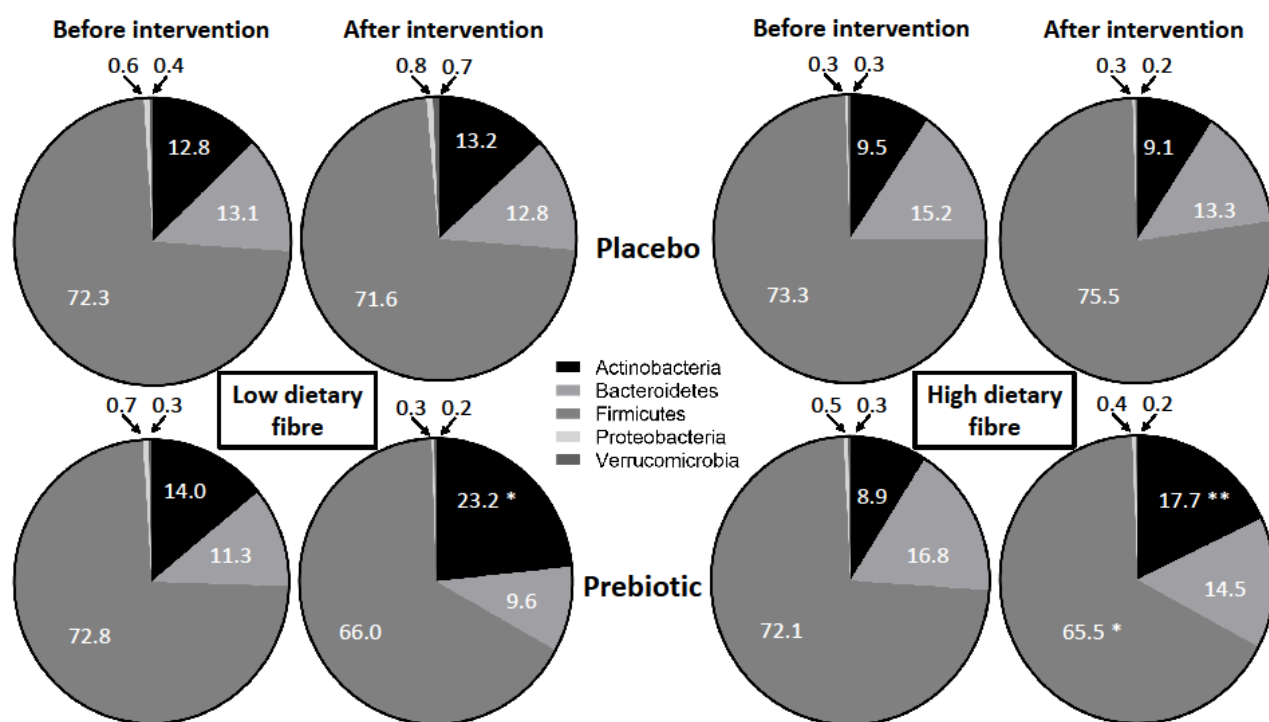


Figure 4

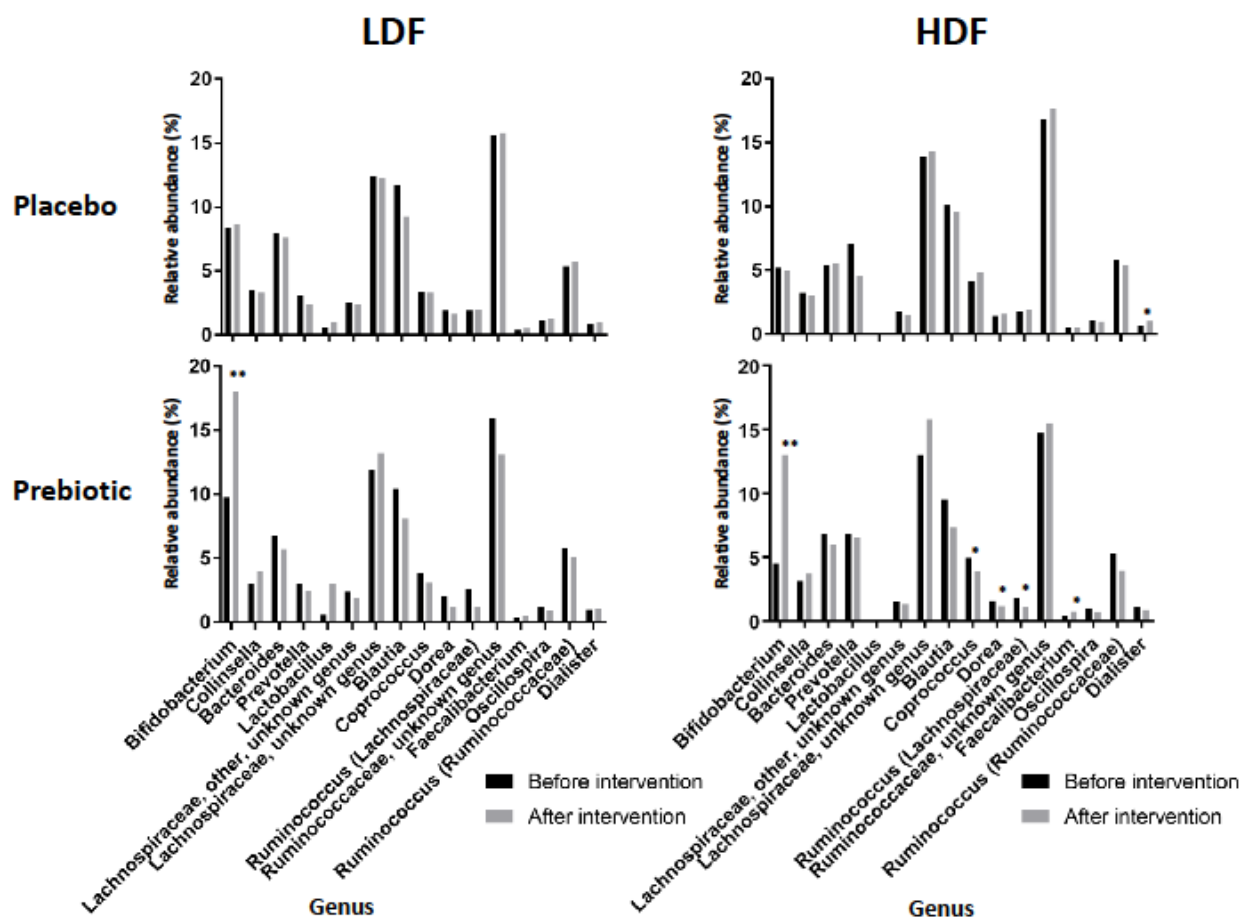
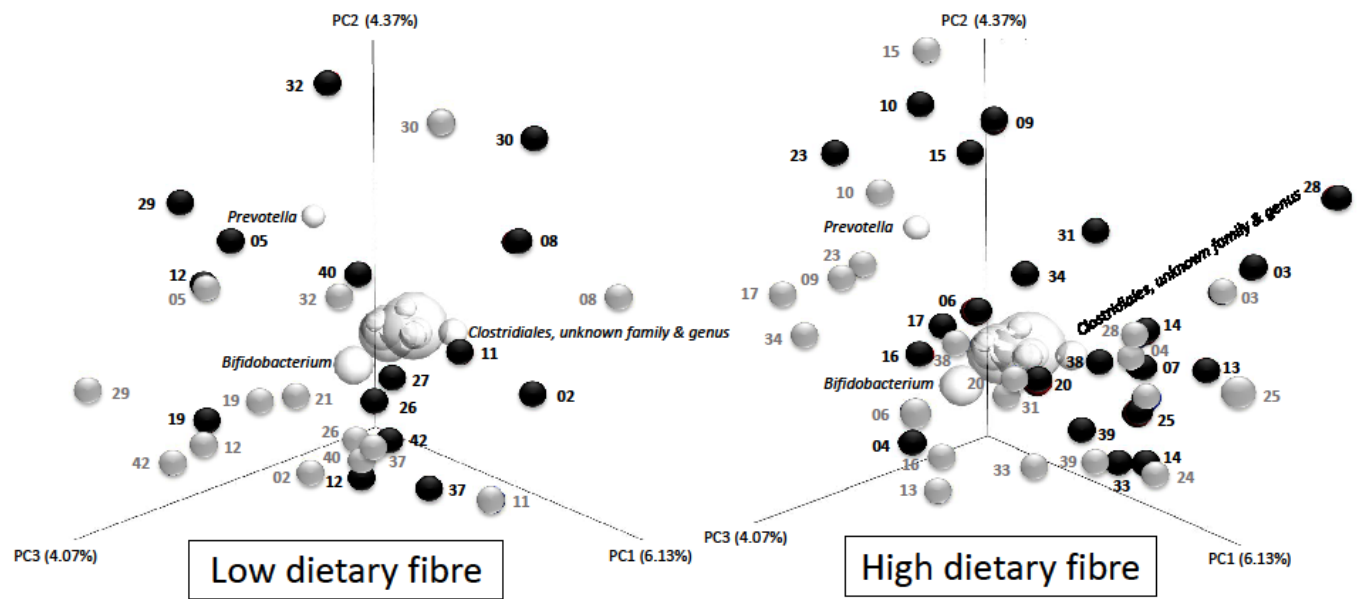


Figure 5

Figure 6



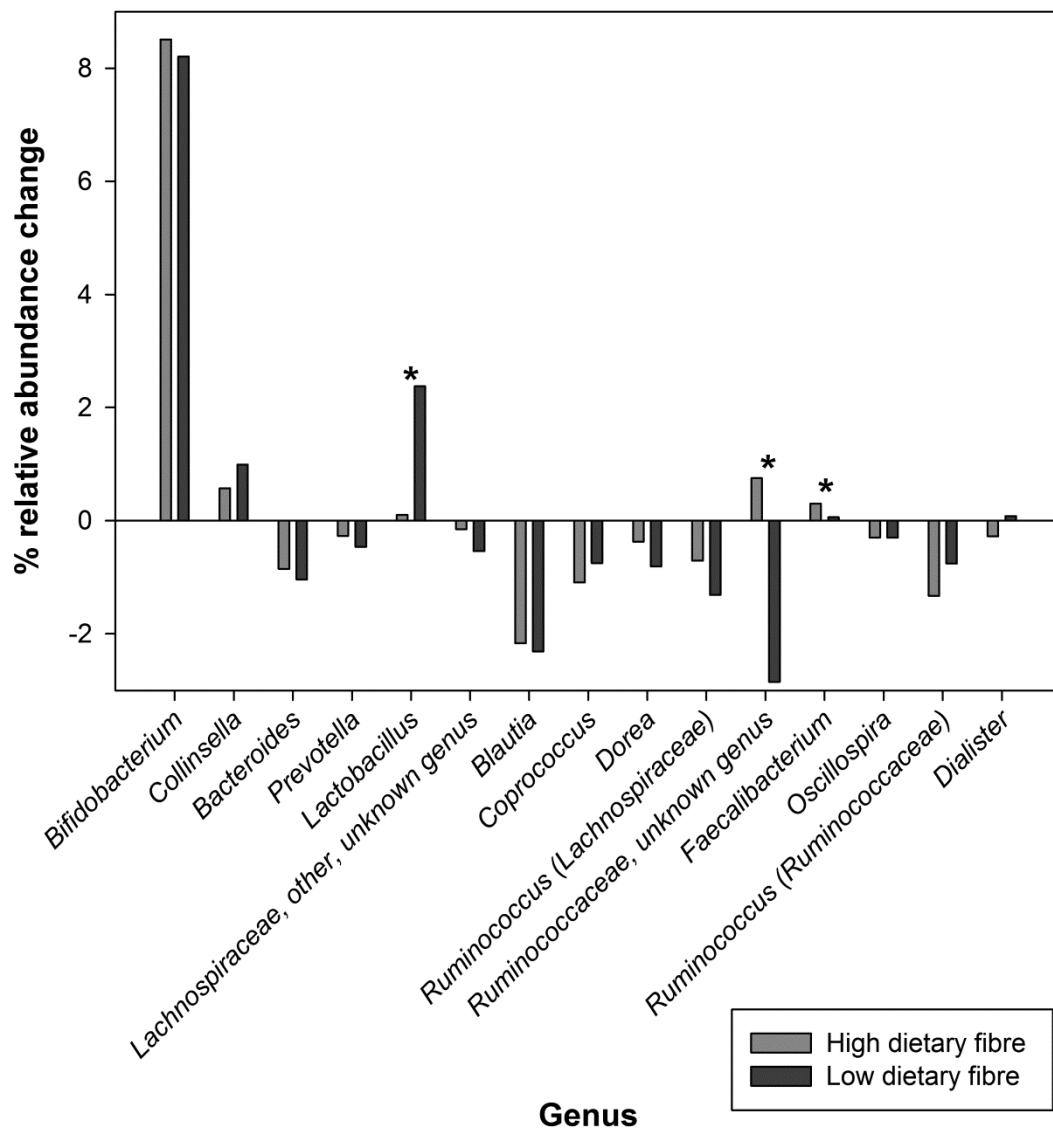


Figure 7

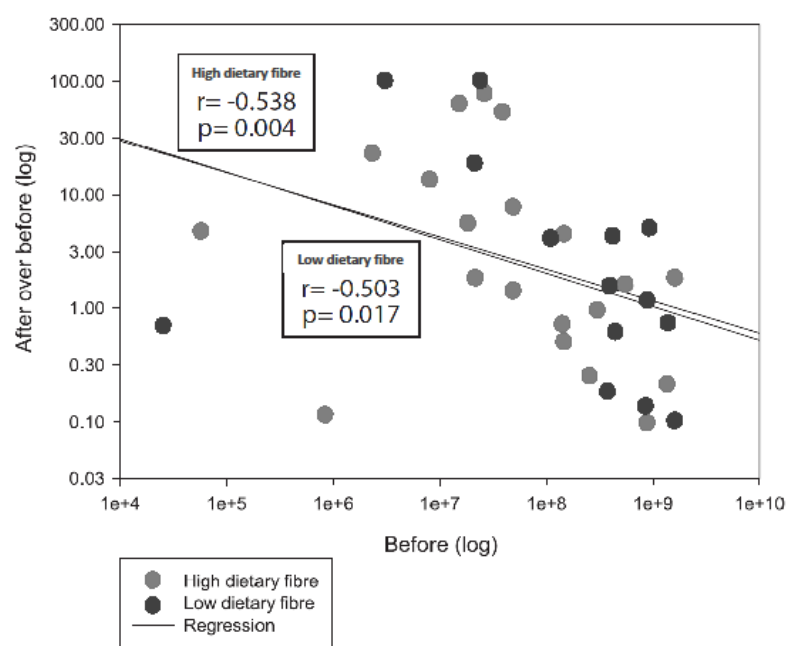


Figure 8

## Online supplemental material

**SUPPLEMENTAL TABLE 1**  
Comparison of dietary intakes throughout the course of the study in the whole cohort<sup>1</sup>

Comparison of dietary intakes throughout the 6-week trial in the whole cohort									
Dietary intake	Prebiotic (n = 34)				Placebo (n = 33)				P value
	Before intervention		After intervention		Before intervention		After intervention		
	Mean	SD	Mean	SD	Mean	SD	Mean	SD	
Energy (kJ/d)	8839.2	2916.4	9120.5	3067	9042.2	2800	8825.3	2284.3	0.963
Protein (g/d)	100.5	41.6	100.4	47.5	100.3	38.8	96.1	32.9	0.963
Total fat (g/d)	84.3	31.2	89.9	39.2	93.6	39.5	88.5	35.7	0.775
Saturated fat (g/d)	30.5	14.3	34.2	16.3	32.9	14.4	33.5	14.1	0.757
Polyunsaturated fat (g/d)	14	5.9	13.6	6.6	15.7	7.2	14	7.1	0.599
Monounsaturated fat (g/d)	32.4	12	34.3	17.2	37.2	18.7	33.2	14.9	0.642
Carbohydrate (g/d)	215.2	88.2	222.7	89.1	209.5	75.8	215.1	69.1	0.93
Sugars (g/d)	94.5	44.8	94.8	36.7	94.4	43.8	98	40.4	0.983
Starch (g/d)	118.5	54.3	125.7	65.2	113.4	49.2	115.8	44.5	0.808
Dietary fibre (g/d)	30.1	14.4	28.2	12.2	30.3	14	28	11.9	0.845
Water (g/d)	2479.5	1236.1	2467.7	1219.7	2523.8	1251.6	2525.5	1368	0.997
Alcohol (g/d)	3.8	10.3	3.2	7.3	2.9	7.5	1.9	3.8	0.779
Energy from protein (%)	19.7	6.1	18.5	5.4	19.4	6.8	18.7	5	0.814
Energy from fat (%)	35.3	7.3	35.8	8.9	37.4	7.8	36.6	9	0.733
Energy from saturated fat (%)	12.7	4.2	13.7	4.3	13.3	4	13.8	3.8	0.628
Energy from carbohydrate (%)	39.8	8.7	40.8	9.3	38.6	8.3	40.5	10.5	0.76
Energy from alcohol (%)	1.1	2.6	1	2.4	0.9	1.9	0.6	1.4	0.808
Energy from fibre (%)	2.7	0.9	2.6	0.9	2.7	0.9	2.5	0.8	0.702

<sup>1</sup> One-way repeated measures ANOVA. P value <0.05 is considered significant. SD: standard deviation

**SUPPLEMENTAL TABLE 2**  
Alpha diversity comparisons at baseline (before intervention), and before and after the prebiotic intervention in the low and high dietary fibre groups<sup>1</sup>

	Low dietary fibre (n=14)					High dietary fibre (n=20)					P value <sup>c</sup>
	Before intervention		After intervention		P value <sup>#</sup>	Before intervention		After intervention		P value <sup>#</sup>	
	Mean	SD	Mean	SD		Mean	SD	Mean	SD		
Alpha diversity index											
Observed species (OTUs)	801.1	156.2	841.7	147.7	0.531	841.5	133.5	836.2	160.3	0.911	0.442
Shannon index	6.08	0.48	5.87	0.45	0.263	6.28	0.50	5.99 <sup>a</sup>	0.47	0.060	0.261
PD whole tree	83.61	15.79	87.50	13.12	0.512	87.30	14.22	86.14	13.74	0.794	0.487
Chao index	5655	817	6977 <sup>a</sup>	2300	0.060	6624	1640	5783	1516	0.100	0.796

<sup>1</sup> Non-parametric two-sample t-test (using QIIME). Mean values are significant different between the low and high dietary fibre groups at baseline (\*, before versus before) and from the before intervention sample within a dietary fibre group (\*, before versus after). <sup>2</sup> P < 0.05, <sup>^</sup> trend towards significance (p < 0.1). SD: standard deviation

SUPPLEMENTAL TABLE 3

Gastrointestinal symptom differences between the placebo and prebiotic intervention phase for the whole cohort and the low and high dietary fibre groups<sup>1</sup>

	Whole cohort			Low dietary fibre			High dietary fibre		
	Placebo	Prebiotic	P value	Placebo	Prebiotic	P value	Placebo	Prebiotic	P value
	(n = 33)	(n = 34)		(n = 14)	(n = 14)		(n = 19)	(n = 20)	
	%	%		%	%		%	%	
<b>Mild (total)</b>	79	79	1.000	79	64	0.687	79	89	0.625
Nausea	9	9	1.000	0	0	1.000	16	16	1.000
Diarrhoea	21	18	1.000	21	0	0.250	21	32	0.687
Flatulence	61	70	0.375	57	50	1.000	63	84	0.125
Gurgling	42	52	0.549	43	43	1.000	42	58	0.375
Cramps	30	27	1.000	43	14	0.219	21	37	0.375
Pain	27	33	0.754	14	21	1.000	37	42	1.000
Bloating	39	45	0.687	29	21	1.000	47	63	0.375
<b>Moderate (total)</b>	12	42	<b>0.013</b>	14	21	1.000	11	58	<b>0.004</b>
Nausea	0	3	1.000	0	7	1.000	0	0	1.000
Diarrhoea	6	0	0.500	7	0	1.000	5	0	1.000
Flatulence	3	30	<b>0.012</b>	7	21	0.625	0	37	<b>0.016</b>
Gurgling	3	6	1.000	0	0	1.000	5	11	1.000
Cramps	0	3	1.000	0	0	1.000	0	5	1.000
Pain	0	15	0.062	0	0	1.000	0	26	0.062
Bloating	3	12	0.375	0	7	1.000	5	16	0.625
<b>Severe (total)</b>	0	6	0.500	0	7	1.000	0	5	1.000
Nausea	0	0	1.000	0	0	1.000	0	0	1.000
Diarrhoea	0	3	1.000	0	0	1.000	0	5	1.000
Flatulence	0	3	1.000	0	7	1.000	0	0	1.000
Gurgling	0	3	1.000	0	0	1.000	0	5	1.000
Cramps	0	0	1.000	0	0	1.000	0	0	1.000
Pain	0	0	1.000	0	0	1.000	0	0	1.000
Bloating	0	0	1.000	0	0	1.000	0	0	1.000

<sup>1</sup>McNemar test. Significant results ( $p < 0.05$ ) are in **bold**. %: the percentage of participants who experienced mild (nagging or annoying), moderate (strong negative influence on daily living) and severe (disabling) gastrointestinal symptoms at least once during the placebo and prebiotic intervention phases. SD: standard deviation



**SUPPLEMENTAL TABLE 4**

Appetite rating (fullness, hunger, much [how much can you eat], satisfaction ratings using an 100 mm anchored visual analogue scale) changes 30 mins before and 30 mins after breakfast, lunch and dinner during the placebo and prebiotic intervention phases in the whole cohort<sup>1</sup>

	Placebo (n = 33)				Prebiotic (n = 34)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Before Breakfast</b>								
Fullness	31.7	14.0	31.2	14.1	31.0	18.4	31.1	17.4
Hunger	52.1	17.3	53.3	20.0	53.2	19.9	50.2	22.1
Much	59.2	16.4	58.7	17.6	58.1	19.3	57.1	18.2
Satisfaction	34.4	12.2	34.4	14.5	37.0	17.9	35.5	18.8
<b>After Breakfast</b>								
Fullness	70.7	13.0	71.4	15.9	68.3	14.7	70.6	19.2
Hunger	21.1	13.5	20.2	13.1	23.3	15.6	20.3	14.2
Much	30.3	15.0	28.1	15.8	28.4	16.4	27.0	15.2
Satisfaction	72.5	12.6	73.8	15.3	70.3	15.3	71.1	18.1
<b>Before Lunch</b>								
Fullness	32.7	13.2	33.1	16.4	30.4	14.3	30.1	15.9
Hunger	57.9	15.9	60.1	19.6	59.0	18.3	61.8	18.2
Much	61.1	15.8	62.0	19.6	63.0	16.0	65.6	15.8
Satisfaction	35.9	12.0	36.1	15.7	34.8	17.2	31.6	16.0
<b>After Lunch</b>								
Fullness	71.3	12.7	68.6	14.3	71.5	13.5	73.8	14.0
Hunger	19.8	13.1	19.9	12.7	21.8	14.2	19.3	11.7
Much	27.9	13.9	28.3	16.1	28.1	18.5	24.3	16.2
Satisfaction	74.2	12.4	71.3	14.3	71.3	14.0	74.8	15.2
<b>Before Dinner</b>								
Fullness	32.3	11.1	30.7	13.5	30.6	14.9	28.5	14.3
Hunger	61.5	13.2	62.1	16.7	59.9	16.8	62.8	18.6
Much	66.1	14.1	66.4	15.7	64.4	16.2	68.8	15.7
Satisfaction	33.6	11.8	33.1	12.1	33.5	14.4	29.9	15.3
<b>After Dinner</b>								
Fullness	78.5	17.0	76.6	14.4	76.8	13.2	78.4	15.2
Hunger	13.1	9.1	17.2*	11.1	17.3	13.6	16.0	9.3
Much	19.8	11.3	22.1	12.8	20.3	13.1	19.2	11.4
Satisfaction	79.2	16.8	78.2	14.1	77.5	15.7	79.7	12.7

<sup>1</sup> Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean value is significantly different from before intervention within a particular intervention phase; \* p < 0.05. SD: standard deviation

# SUPPLEMENTAL TABLE 5

Appetite rating (full, hungry, much [how much can you eat], satisfied ratings using an 100 mm anchored visual analogue scale) changes 30 mins before and 30 mins after breakfast, lunch and dinner during the placebo and prebiotic intervention phases in the low dietary fibre group<sup>1</sup>

	Placebo (n = 14)				Prebiotic (n = 14)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Before Breakfast</b>								
Fullness	32.1	13.0	34.1	13.2	35.5	19.6	33.9	13.9
Hunger	56.0	15.5	54.1	15.9	47.2	19.0	46.5	18.6
Much	59.9	16.9	56.6	14.6	51.8	17.9	54.4	14.5
Satisfaction	32.2	11.8	33.4	11.6	39.6	18.5	36.3	16.2
<b>After Breakfast</b>								
Fullness	70.5	15.2	72.8	17.0	69.4	18.2	65.4	24.5
Hunger	21.5	13.9	23.2	14.5	25.2	21.1	24.8	14.2
Much	29.8	18.9	30.3	18.7	27.0	20.0	29.7	18.4
Satisfaction	71.1	14.9	72.1	17.6	71.4	18.2	67.2	23.4
<b>Before Lunch</b>								
Fullness	38.5	10.6	36.1	19.6	32.2	15.8	35.4	18.8
Hunger	50.5	17.0	53.6	23.3	58.0	19.7	57.3	17.6
Much	54.6	16.6	54.4	22.7	61.4	16.3	62.2	14.5
Satisfaction	42.8	10.3	38.4	18.3	32.6	16.5	35.6	17.5
<b>After Lunch</b>								
Fullness	71.0	13.2	75.5	15.3	73.3	17.1	73.8	15.4
Hunger	21.1	16.1	18.1	11.3	20.4	17.4	18.9	13.6
Much	25.1	15.6	22.1	14.3	25.2	22.1	23.2	19.6
Satisfaction	74.1	13.0	75.1	15.5	74.2	15.8	75.0	16.3
<b>Before Dinner</b>								
Fullness	32.3	11.5	34.6	15.5	33.0	17.1	30.1	17.5
Hunger	62.1	14.6	59.3	20.4	57.0	19.2	61.7	20.5
Much	62.3	15.6	60.7	17.8	62.1	17.1	63.9	19.3
Satisfaction	35.0	13.4	36.0	13.9	33.4	15.3	30.9	18.1
<b>After Dinner</b>								
Fullness	82.2	19.1	80.5	18.6	83.2	10.2	78.8	20.0
Hunger	13.7	10.8	14.5	10.4	13.1	11.0	16.2	12.2
Much	18.0	13.9	17.2	13.9	15.4	10.3	17.2	13.7
Satisfaction	80.3	20.2	80.3	19.5	83.4	10.0	79.8	17.0

<sup>1</sup> Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean values were not significantly different ( $p > 0.05$ ) from each other. SD: standard deviation

**SUPPLEMENTAL TABLE 6**

Appetite rating (full, hungry, much [how much can you eat], satisfied ratings using an 100 mm anchored visual analogue scale) changes 30 mins before and 30 mins after breakfast, lunch and dinner during the placebo and prebiotic intervention phases in the high dietary fibre group<sup>1</sup>

	Placebo (n = 19)				Prebiotic (n = 20)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Before Breakfast</b>								
Fullness	31.4	15.1	29.0	14.7	27.7	17.1	29.1	19.6
Hunger	49.3	18.4	51.6	23.1	57.5	19.9	52.8	24.5
Much	58.7	16.5	61.2	19.9	62.7	19.5	59.1	20.7
Satisfaction	36.0	12.5	35.3	16.8	35.1	17.8	34.8	21.0
<b>After Breakfast</b>								
Fullness	70.9	11.4	71.1	15.4	67.5	12.0	74.4	13.7
Hunger	20.7	13.7	17.0	11.7	21.9	10.3	17.0	13.6
Much	30.6	12.0	25.9	13.4	29.4	13.7	25.0	12.4
Satisfaction	73.6	10.9	75.8	13.6	69.5	13.4	74.1	12.8
<b>Before Lunch</b>								
Fullness	28.6	13.6	30.4	13.7	29.2	13.5	26.4	12.8
Hunger	63.1	13.2	64.7	15.2	59.7	17.8	64.9	18.4
Much	65.7	13.9	68.5	15.3	64.2	16.0	67.9	16.6
Satisfaction	31.1	10.9	33.8	13.9	36.3	17.9	28.8*	14.7
<b>After Lunch</b>								
Fullness	71.5	12.7	64.3**	11.4	70.4	10.6	73.9**	13.4
Hunger	18.9	11.0	20.5	13.8	22.8	11.9	19.6	10.6
Much	29.9	12.7	32.0	16.2	30.1	15.7	25.1	13.8
Satisfaction	74.2	12.2	69.0*	13.1	69.3	12.5	74.7*	14.8
<b>Before Dinner</b>								
Fullness	32.3	11.2	28.1	11.4	28.9	13.3	27.5	12.0
Hunger	61.1	12.5	63.4	13.6	62.0	15.0	63.6	17.6
Much	68.8	12.6	70.6	12.8	66.0	15.8	72.2	12.0
Satisfaction	32.6	10.8	30.9	10.4	33.6	14.2	29.2	13.5
<b>After Dinner</b>								
Fullness	75.9	15.2	74.5	10.0	72.4	13.5	78.2	11.4
Hunger	12.4	7.9	19.2**	11.5	20.3	14.8	15.8**	7.1
Much	21.0	9.1	25.5	10.9	23.7	14.0	20.6	9.5
Satisfaction	78.4	14.3	76.6	8.7	73.3	17.8	79.6	9.1

<sup>1</sup> Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean values are significantly different from before intervention within a particular intervention phase; \* p < 0.05, \*\* p < 0.01. SD: standard deviation

SUPPLEMENTAL TABLE 7

Short-chain fatty acid concentration and bacterial taxa changes during the placebo and prebiotic intervention phases in the low dietary fibre group<sup>1</sup>

	Low dietary fibre							
	Placebo (n = 14)				Prebiotic (n = 14)			
	Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention	Before intervention	After intervention
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Short-chain fatty acids (μmol/g)</b>								
Acetate	31.46	18.07	33.64	17.90	28.97	18.23	34.26	22.33
Butyrate	10.34	7.16	9.38	5.42	7.77	5.10	8.27	5.28
Propionate	10.02	5.76	12.62	8.30	9.99	6.31	10.88	7.08
Sum of short-chain fatty acids	55.73	28.84	61.43	32.34	51.02	29.00	57.59	33.95
<b>Phylum (% relative abundance)</b>								
Actinobacteria	12.81	6.11	13.16	7.99	13.98	9.42	23.17**	9.59
Bacteroidetes	13.07	8.06	12.84	5.33	11.31	8.94	9.56	5.43
Firmicutes	72.30	7.36	71.59	9.73	72.82	8.79	65.96	9.74
Proteobacteria	0.62	0.53	0.78	0.85	0.65	0.93	0.30^	0.36
Verrucomicrobia	0.41	0.69	0.68	1.18	0.33	0.58	0.20	0.46
<b>Genus (% relative abundance)</b>								
<i>Bifidobacterium</i>	8.40	5.53	8.62	6.83	9.81	7.78	18.01**	7.85
<i>Collinsella</i>	3.51	2.64	3.30	3.13	2.95	3.09	3.94	3.22
<i>Bacteroides</i>	7.98	3.82	7.63	4.38	6.77	5.01	5.73	3.91
<i>Prevotella</i>	3.08	5.50	2.39	3.62	2.94	4.89	2.48	3.62
<i>Lactobacillus</i>	0.56	1.38	1.02	2.98	0.59	1.29	2.97	5.64
<i>Lachnospiraceae</i> , other, unknown genus	2.50	1.31	2.41	1.58	2.38	1.46	1.84	0.64
<i>Lachnospiraceae</i> , unknown genus	12.37	7.68	12.28	5.14	11.85	7.20	13.24	7.36
<i>Blautia</i>	11.72	7.02	9.25	4.33	10.42	5.57	8.10	4.08
<i>Coprococcus</i>	3.43	1.82	3.31	1.88	3.83	2.13	3.07	1.87
<i>Dorea</i>	1.93	1.01	1.60	0.85	2.01	1.22	1.19	0.54
<i>Ruminococcus (Lachnospiraceae)</i>	1.91	2.09	1.94	1.74	2.55	1.99	1.24^	1.31
<i>Ruminococcaceae</i> , unknown genus	15.63	5.19	15.78	4.38	15.95	5.08	13.10^	4.84
<i>Faecalibacterium</i>	0.42	0.39	0.51	0.34	0.39	0.29	0.45	0.33
<i>Oscillospira</i>	1.10	0.62	1.25	0.84	1.21	0.65	0.91^	0.47
<i>Ruminococcus (Ruminococcaceae)</i>	5.35	3.28	5.67	4.39	5.80	4.26	5.04	4.01
<i>Dialister</i>	0.86	1.36	0.97	1.71	1.00	1.65	1.09	2.13

<sup>1</sup> Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean values are significantly different from those of the placebo intervention and prebiotic before intervention; \*p < 0.05, \*\*p < 0.01, ^trend towards significance (p < 0.1). SD: standard deviation

SUPPLEMENTAL TABLE 8

Short-chain fatty acid concentration and bacterial taxa changes during the placebo and prebiotic intervention phases in the high dietary fibre group<sup>1</sup>

	High dietary fibre							
	Placebo (n = 19)				Prebiotic (n = 20)			
	Before intervention		After intervention		Before intervention		After intervention	
	Mean	SD	Mean	SD	Mean	SD	Mean	SD
<b>Short-chain fatty acids (μmol/g)</b>								
Acetate	32.02	18.08	33.92	20.21	33.32	19.74	43.17	19.69
Butyrate	9.32	5.39	9.49	5.92	9.08	5.80	11.48	5.60
Propionate	10.14	6.64	10.90	7.52	10.05	8.90	12.69	7.83
Sum of short-chain fatty acids	55.37	29.37	58.05	33.05	56.57	33.12	71.06	31.05
<b>Phylum (% relative abundance)</b>								
Actinobacteria	9.45	6.45	9.13	6.32	8.87	5.97	17.70**	10.23
Bacteroidetes	15.20	14.53	13.27	10.23	16.82	11.44	14.50	9.48
Firmicutes	73.34	13.71	75.52	11.85	72.12	12.15	65.54*	12.09
Proteobacteria	0.30	0.21	0.32	0.22	0.47	0.47	0.41	0.45
Verrucomicrobia	0.27	0.66	0.16	0.41	0.26	0.33	0.15	0.21
<b>Genus (% relative abundance)</b>								
<i>Bifidobacterium</i>	5.20	4.65	4.94	4.65	4.51	4.10	13.02**	8.58
<i>Collinsella</i>	3.25	2.50	3.05	2.62	3.15	2.69	3.72	2.53
<i>Bacteroides</i>	5.38	3.49	5.58	4.16	6.81	3.66	5.96	3.10
<i>Prevotella</i>	7.05	15.29	4.60	7.20	6.79	11.03	6.51	9.73
<i>Lactobacillus</i>	0.01	0.01	0.02	0.04	0.03	0.06	0.07	0.24
<i>Lachnospiraceae</i> , other, unknown genus	1.75	0.82	1.54	0.78	1.50	0.78	1.35	0.54
<i>Lachnospiraceae</i> , unknown genus	13.94	3.99	14.27	5.94	13.04	5.58	15.80	5.39
<i>Blautia</i>	10.09	4.82	9.59	4.61	9.53	4.36	7.36	3.82
<i>Coproccoccus</i>	4.08	1.84	4.79	2.26	4.97	2.74	3.88*	1.42
<i>Dorea</i>	1.44	0.69	1.62	0.89	1.57	0.71	1.20*	0.75
<i>Ruminococcus (Lachnospiraceae)</i>	1.80	1.31	1.95	1.61	1.81	1.20	1.10*	0.83
<i>Ruminococcaceae</i> , unknown genus	16.84	4.60	17.65	4.15	14.74	3.12	15.49	3.31
<i>Faecalibacterium</i>	0.50	0.27	0.56	0.28	0.42	0.18	0.72*	0.28
<i>Oscillospira</i>	1.10	0.72	0.99	0.59	1.00	0.42	0.70	0.45
<i>Ruminococcus (Ruminococcaceae)</i>	5.78	4.11	5.41	3.80	5.27	3.47	3.94	2.77
<i>Dialister</i>	0.70	1.00	1.03*	1.48	1.12	1.81	0.84	1.14

<sup>1</sup> Two-way repeated-measures ANOVA (blocked by participant) and least significant difference test. Mean values are significantly different from those of the placebo intervention and prebiotic before intervention or in the case of *Dialister* significantly different from those of the prebiotic intervention and placebo before intervention; \*p < 0.05, \*\*p < 0.01. SD: standard deviation